

WHAT IS CLAIMED IS:

1. A reverse transcriptase which has been modified or mutated to increase or enhance thermostability.
2. The reverse transcriptase of claim 1, wherein the reverse transcriptase has one or more modifications or mutations at positions corresponding to amino acids selected from the group consisting of:
  - (a) leucine 52 of M-MLV reverse transcriptase;
  - (b) tyrosine 64 of M-MLV reverse transcriptase;
  - (c) lysine 152 of M-MLV reverse transcriptase;
  - (d) histidine 204 of M-MLV reverse transcriptase;
  - (e) methionine 289 of M-MLV reverse transcriptase;
  - (f) threonine 306 of M-MLV reverse transcriptase; and
  - (g) phenylalanine 309 of M-MLV reverse transcriptase.
3. The reverse transcriptase of claim 2, which is M-MLV reverse transcriptase.
4. The reverse transcriptase of claim 3, wherein leucine 52 is replaced with proline.
5. The reverse transcriptase of claim 3, wherein tyrosine 64 is replaced with arginine.
6. The reverse transcriptase of claim 3, wherein lysine 152 is replaced with methionine.
7. The reverse transcriptase of claim 3, wherein histidine 204 is replaced with arginine.
8. The reverse transcriptase of claim 3, wherein methionine 289 is replaced with leucine.

9. The reverse transcriptase of claim 3, wherein threonine 306 is replaced with either lysine or arginine.

10. The reverse transcriptase of claim 3, wherein the reverse transcriptase has a mutation or modification at amino acids histidine 204 and threonine 306.

11. The reverse transcriptase of claim 10, wherein histidine 204 is replaced with arginine and threonine 306 is replaced with either lysine or arginine.

12. The reverse transcriptase of claim 1, which retains at least 50% of reverse transcriptase activity after heating to 50°C for 5 minutes.

13. The reverse transcriptase of claim 1, which retains at least 70% of reverse transcriptase activity after heating to 50°C for 5 minutes.

14. The reverse transcriptase of claim 1, which retains at least 85% of reverse transcriptase activity after heating to 50°C for 5 minutes.

15. The reverse transcriptase of claim 1, which retains at least 95% of reverse transcriptase activity after heating to 50°C for 5 minutes.

16. The reverse transcriptase of claim 1, wherein the reverse transcriptase has one or more properties selected from the group consisting of:

- (a) reduced or substantially reduced RNase H activity;
- (b) reduced or substantially reduced terminal deoxynucleotidyl transferase activity; and
- (c) increased fidelity.

17. The reverse transcriptase of claim 16, wherein the reverse transcriptase has reduced or substantially reduced RNase H activity.

18. The reverse transcriptase of claim 16, wherein the reverse transcriptase has reduced or substantially reduced terminal deoxynucleotidyl transferase activity.

19. The reverse transcriptase of claim 18, wherein the reverse transcriptase has one or more one or more modifications or mutations at positions corresponding to amino acids selected from the group consisting of:

- (a) tyrosine 133 of M-MLV reverse transcriptase;
- (b) threonine 197 of M-MLV reverse transcriptase; and
- (c) phenylalanine 309 of M-MLV reverse transcriptase.

20. The reverse transcriptase of claim 19, which is M-MLV reverse transcriptase.

21. The reverse transcriptase of claim 20, wherein tyrosine 133 is replaced with alanine.

22. The reverse transcriptase of claim 20, wherein threonine 197 is replaced with glutamic acid.

23. The reverse transcriptase of claim 20, wherein phenylalanine 309 is replaced with asparagine.

24. The reverse transcriptase of claim 16, wherein the reverse transcriptase has increased fidelity.

25. The reverse transcriptase of claim 24, wherein the reverse transcriptase has one or more one or more modifications or mutations at positions corresponding to amino acids selected from the group consisting of:

- (a) tyrosine 64 of M-MLV reverse transcriptase;
- (b) arginine 116 of M-MLV reverse transcriptase;
- (c) glutamine 190 of M-MLV reverse transcriptase; and
- (d) valine 223 of M-MLV reverse transcriptase.

26. The reverse transcriptase of claim 1, wherein the reverse transcriptase is selected from the group consisting of M-MLV, RSV, AMV, and HIV reverse transcriptases.

27. The reverse transcriptase of claim 26, wherein the reverse transcriptase is selected from the group consisting of M-MLV RNase H- reverse transcriptase, RSV RNase H- reverse transcriptase, AMV RNase H- reverse transcriptase, RAV RNase H- reverse transcriptase, and HIV RNase H- reverse transcriptase.

28. The reverse transcriptase of claim 26, wherein the reverse transcriptase is an M-MLV reverse transcriptase.

29. The reverse transcriptase of claim 28, wherein aspartic acid 524 is replaced with glycine, glutamic acid 562 is replaced with glutamine, and aspartic acid 583 is replaced with asparagine.

30. A vector comprising nucleic acid encoding the reverse transcriptase of claim 1.

31. The vector of claim 30, wherein the nucleic acid is operably linked to a promoter.

32. The vector of claim 31, wherein the promoter is selected from the group consisting of a  $\lambda$ -P<sub>L</sub> promoter, a *tac* promoter, a *trp* promoter, an ara BAD promoter and a *trc* promoter.

33. A host cell comprising the vector of claim 30.

34. A method of producing a reverse transcriptase, the method comprising:

(a) culturing the host cell of claim 33;

- (b) expressing the nucleic acid; and
- (c) isolating the reverse transcriptase from the host cell.

35. The method of claim 34, wherein the host cell is an *Escherichia coli*.

36. A method for reverse transcription of one or more nucleic acid molecules comprising:

- (a) mixing one or more nucleic acid templates with one or more reverse transcriptases of claim 1; and
- (b) incubating the mixture of (a) under conditions sufficient to make one or more first nucleic acid molecules complementary to all or a portion of the one or more templates.

37. The method of claim 36, wherein the nucleic acid template is a messenger RNA molecule or a population of mRNA molecules.

38. The method of claim 37, the method further comprising the step of incubating the one or more first nucleic acid molecules under conditions sufficient to make one or more second nucleic acid molecules complementary to all or a portion of the one or more first nucleic acid molecules.

39. A cDNA molecule made according to the method of claim 36.

40. A cDNA molecule made according to the method of claim 38.

41. A method for amplifying one or more nucleic acid molecules, the method comprising:

- (a) mixing one or more nucleic acid templates with one or more reverse transcriptases of claim 1 and one or more DNA polymerases; and

(b) incubating the mixture of (a) under conditions sufficient to amplify one or more nucleic acid molecules complementary to all or a portion of the one or more templates.

42. A method for sequencing one or more nucleic acid molecules, the method comprising:

(a) mixing one or more nucleic acid molecules to be sequenced with one or more primers, one or more reverse transcriptases of claim 1, one or more nucleotides and one or more terminating agents;

(b) incubating the mixture of (a) under conditions sufficient to synthesize a population of molecules complementary to all or a portion of the one or more molecules to be sequenced; and

(c) separating the population to determine the nucleotide sequence of all or a portion of the one or more molecules to be sequenced.

43. A method for sequencing a nucleic acid molecule, the method comprising:

(a) mixing a nucleic acid molecules to be sequenced with one or more primers, one or more reverse transcriptases of claim 1, one or more nucleotides and one or more terminating agents;

(b) incubating the mixture of (a) under conditions sufficient to synthesize a population of molecules complementary to all or a portion of the molecule to be sequenced; and

(c) separating members of the population to determine the nucleotide sequence of all or a portion of the molecule to be sequenced.

44. A kit for use in reverse transcription, amplification or sequencing of a nucleic acid molecule, the kit comprising one or more reverse transcriptases of claim 1.

45. The kit of claim 44, the kit further comprising one or more components selected from the group consisting of one or more nucleotides,

one or more DNA polymerases, a suitable buffer, one or more primers and one or more terminating agents.

46. The kit of claim 45, wherein the terminating agent is a dideoxynucleotide.

47. The kit of claim 44, wherein the reverse transcriptase is an M-MLV reverse transcriptase.

48. The kit of claim 47, wherein the reverse transcriptase has one or more modifications or mutations at positions corresponding to amino acids selected from the group consisting of:

- (a) leucine 52 of M-MLV reverse transcriptase;
- (b) tyrosine 64 of M-MLV reverse transcriptase;
- (c) lysine 152 of M-MLV reverse transcriptase;
- (d) arginine 204 of M-MLV reverse transcriptase;
- (e) methionine 289 of M-MLV reverse transcriptase;
- (f) threonine 306 of M-MLV reverse transcriptase; and
- (g) phenylalanine 309 of M-MLV reverse transcriptase.

49. A modified or mutated reverse transcriptase having an RNA-dependent DNA polymerase activity that has a half-life of greater than 10 minutes at 50°C.

50. The reverse transcriptase of claim 49, which is a retroviral reverse transcriptase.

51. The reverse transcriptase of claim 50 which is selected from a group consisting of M-MLV reverse transcriptase, ASV reverse transcriptase, HIV reverse transcriptase, Avian Sarcoma-Leukosis Virus (ASLV) reverse transcriptase, Rous Sarcoma Virus (RSV) reverse transcriptase, Avian Myeloblastosis Virus (AMV) reverse transcriptase, Avian Erythroblastosis Virus (AEV) Helper Virus MCAV reverse transcriptase, Avian

Myelocytomatosis Virus MC29 Helper Virus MCAV reverse transcriptase, Avian Reticuloendotheliosis Virus (REV-T) Helper Virus REV-A reverse transcriptase, Avian Sarcoma Virus UR2 Helper Virus UR2AV reverse transcriptase, Avian Sarcoma Virus Y73 Helper Virus YAV reverse transcriptase, Rous Associated Virus (RAV) reverse transcriptase, Myeloblastosis Associated Virus (MAV) reverse transcriptase, and fragments of any of the above having reverse transcriptase activity.

52. The reverse transcriptase of claim 49, wherein the half-life is greater than about 100 minutes.

53. The reverse transcriptase of claim 49, wherein the half-life is greater than about 200 minutes.

54. A modified or mutated reverse transcriptase having a reverse transcriptase activity that has a half-life of greater than about 2 minutes at 55°C.

55. The reverse transcriptase of claim 54, which is a retroviral reverse transcriptase.

56. The reverse transcriptase of claim 55, which is selected from a group consisting of M-MLV reverse transcriptase, ASV reverse transcriptase, HIV reverse transcriptase, Avian Sarcoma-Leukosis Virus (ASLV) reverse transcriptase, Rous Sarcoma Virus (RSV) reverse transcriptase, Avian Myeloblastosis Virus (AMV) reverse transcriptase, Avian Erythroblastosis Virus (AEV) Helper Virus MCAV reverse transcriptase, Avian Myelocytomatosis Virus MC29 Helper Virus MCAV reverse transcriptase, Avian Reticuloendotheliosis Virus (REV-T) Helper Virus REV-A reverse transcriptase, Avian Sarcoma Virus UR2 Helper Virus UR2AV reverse transcriptase, Avian Sarcoma Virus Y73 Helper Virus YAV reverse transcriptase, Rous Associated Virus (RAV) reverse transcriptase,

Myeloblastosis Associated Virus (MAV) reverse transcriptase, and fragments of any of the above having reverse transcriptase activity.

57. The reverse transcriptase of claim 54, wherein the half-life is greater than about 5 minutes.

58. A reverse transcriptase that synthesizes an amount of full length product, wherein the amount of full length product synthesized at 50°C is at least 50% or more of the amount of full length product it synthesizes at 40°C.

59. The reverse transcriptase of claim 58, wherein the amount of full length product synthesized at 50°C is at least 70% or more of the amount of full length product it synthesizes at 40°C.

60. The reverse transcriptase of claim 58, wherein the amount of full length product synthesized at 50°C is at least 80% or more of the amount of full length product it synthesizes at 40°C.

61. The reverse transcriptase of claim 58, wherein the amount of full length product synthesized at 50°C is at least 90% or more of the amount of full length product it synthesizes at 40°C.

62. A reverse transcriptase that synthesizes an amount of full length product, wherein the amount of full length product synthesized at 52.5°C is at least 20% or more of the amount of full length product it synthesizes at 40°C.

63. The reverse transcriptase of claim 62, wherein the amount of full length product synthesized at 52.5°C is at least 40% or more of the amount of full length product it synthesizes at 40°C.

64. The reverse transcriptase of claim 62, wherein the amount of full length product synthesized at 52.5°C is at least 50% or more of the amount of full length product it synthesizes at 40°C.

65. The reverse transcriptase of claim 62, wherein the amount of full length product synthesized at 52.5°C is at least 60% or more of the amount of full length product it synthesizes at 40°C.

66. A reverse transcriptase that synthesizes an amount of full length product, wherein the amount of full length product synthesized at 55°C is at least 1% or more of the amount of full length product it synthesizes at 40°C.

67. The reverse transcriptase of claim 66, wherein the amount of full length product synthesized at 55°C is at least 5% or more of the amount of full length product it synthesizes at 40°C.

68. The reverse transcriptase of claim 66, wherein the amount of full length product synthesized at 55°C is at least 10% or more of the amount of full length product it synthesizes at 40°C.

69. The reverse transcriptase of claim 66, wherein the amount of full length product synthesized at 55°C is at least 20% or more of the amount of full length product it synthesizes at 40°C.

70. The reverse transcriptase of any one of claims 58-69, which is a modified or mutated reverse transcriptase.

71. The reverse transcriptase of claim 70, which comprises one or more modifications or mutations corresponding to amino acids selected from the group consisting of:

- (a) leucine 52 of M-MLV reverse transcriptase;
- (b) tyrosine 64 of M-MLV reverse transcriptase;
- (c) lysine 152 of M-MLV reverse transcriptase;
- (d) histidine 204 of M-MLV reverse transcriptase;
- (e) methionine 289 of M-MLV reverse transcriptase;
- (f) threonine 306 of M-MLV reverse transcriptase; and
- (g) phenylalanine 309 of M-MLV reverse transcriptase.

72. The reverse transcriptase of claim 71, which comprises modifications or mutations corresponding to H204R, M289L, T306K, and F309N of M-MLV reverse transcriptase.

73. A modified or mutated thermostable reverse transcriptase, wherein the reverse transcriptase has an increase in thermostability of greater than about 1.5 fold at 50°C compared to a corresponding un-modified or un-mutated reverse transcriptase.

74. The reverse transcriptase of claim 73, wherein the increase in thermostability is measured by comparing an amount of full length product synthesized by the mutated reverse transcriptase to an amount synthesized by the un-modified or un-mutated reverse transcriptase.

75. A modified or mutated thermostable reverse transcriptase, wherein the reverse transcriptase has an increase in thermostability of greater than about 2 fold at 52.5°C compared to a corresponding un-modified or un-mutated reverse transcriptase.

76. The reverse transcriptase of claim 75, wherein the reverse transcriptase has an increase in thermostability of greater than about 5 fold at 52.5°C compared to a corresponding un-modified or un-mutated reverse transcriptase.

77. The reverse transcriptase of claim 75, wherein the reverse transcriptase has an increase in thermostability of greater than about 8 fold at 52.5°C compared to a corresponding un-modified or un-mutated reverse transcriptase.

78. The reverse transcriptase according to claim 75, wherein the increase in thermostability is measured by comparing an amount of full length product synthesized by the mutated reverse transcriptase to an amount synthesized by the un-modified or un-mutated reverse transcriptase.

79. A mutated thermostable reverse transcriptase, wherein the reverse transcriptase has an increase in thermostability of greater than about 5 fold at 55°C compared to a corresponding un-modified or un-mutated reverse transcriptase.

80. The reverse transcriptase of claim 79, wherein the reverse transcriptase has an increase in thermostability of greater than about 10 fold at 55°C compared to a corresponding un-modified or un-mutated reverse transcriptase.

81. The reverse transcriptase of claim 79, wherein the reverse transcriptase has an increase in thermostability of greater than about 50 fold at 55°C compared to a corresponding un-modified or un-mutated reverse transcriptase.

82. The reverse transcriptase of claim 79, wherein the increase in thermostability is measured by comparing an amount of full length product

synthesized by the mutated reverse transcriptase to an amount synthesized by the un-modified or un-mutated reverse transcriptase.

83. A composition comprising a reverse transcriptase which has been modified or mutated to increase or enhance thermostability.

84. The composition of claim 83, wherein the reverse transcriptase has one or more modifications or mutations at positions corresponding to amino acids selected from the group consisting of:

- (a) leucine 52 of M-MLV reverse transcriptase;
- (b) tyrosine 64 of M-MLV reverse transcriptase;
- (c) lysine 152 of M-MLV reverse transcriptase;
- (d) histidine 204 of M-MLV reverse transcriptase;
- (e) methionine 289 of M-MLV reverse transcriptase;
- (f) threonine 306 of M-MLV reverse transcriptase; and
- (g) phenylalanine 309 of M-MLV reverse transcriptase.

85. The composition of claim 83, wherein the reverse transcriptase comprises modifications or mutations corresponding to H204R, M289L, T306K, and F309N of M-MLV reverse transcriptase.

86. The composition of claim 83, further comprising a DNA polymerase.

87. The composition of claim 83, further comprising an mRNA molecule.

88. The composition of claim 83, further comprising one or more nucleoside triphosphates.

89. The composition of claim 88, wherein at least one nucleotide comprises a label.

90. The composition of claim 89, wherein at least one label is a fluorescent label.

91. The composition of claim 83, further comprising  $Mg^{2+}$  and not containing  $Mn^{2+}$ .

92. A method of preparing a labeled nucleic acid, comprising:

(a) hybridizing a primer to a first nucleic acid template molecule to form a complex; and

(b) incubating the complex in the presence of a polypeptide of the invention and one or more deoxyribonucleoside triphosphates under conditions sufficient to synthesize a second nucleic acid molecule complementary to all or a portion of the template, wherein the second nucleic acid molecule comprises at least one modified nucleotide and/or at least one labeled nucleotide.

93. The method of claim 92, wherein the template comprises about 20  $\mu g$  of total RNA or 1  $\mu g$  poly(A)RNA.

94. The method of claim 92, wherein the conditions comprise incubation at a temperature of from about 42°C to about 60°C.

95. The method of claim 94, wherein the conditions comprise incubation at a temperature of from about 50°C to about 55°C.

96. A labeled nucleic acid molecule produced by the method of claim 92.

97. A method of detecting a target nucleic acid sequence, comprising contacting a sample comprising the target sequence with the nucleic acid molecule of claim 96, and detecting the nucleic acid molecule of claim 96.

98. The method of claim 97, wherein from about 1 pg to about 100 pg of target sequence is present in the sample.

99. The method of claim 98, wherein about 10 pg of target sequence is present in the sample.

100. A method according to claim 92, wherein conditions comprise at least one modified nucleotide.

101. The method of claim 101, wherein the template comprises about 5 µg of total RNA or about 0.4 µg poly(A)RNA.

102. The method of claim 100, wherein the conditions comprise incubation at a temperature of from about 42°C to about 60°C.

103. The method of claim 100, wherein the conditions comprise incubation at a temperature of from about 50°C to about 55°C.

104. The method of claim 100, further comprising reacting the second nucleic acid molecule with a dye containing molecule to produce a labeled nucleic acid molecule.

105. A labeled nucleic acid molecule produced by the method of claim 105.

106. A method of detecting a target nucleic acid sequence, comprising contacting a sample comprising the target sequence with the nucleic acid molecule of claim 105, and  
detecting the nucleic acid molecule of claim 105.

107. The method of claim 106, wherein from about 0.1 pg to about 100 pg of target sequence is present in the sample.

108. The method of claim 107, wherein about 2 pg of target sequence is present in the sample.

109. The method of claim 104, wherein conditions result in uniform dye incorporation for both Cy3 and Cy5.